CYTOLOGICAL STUDIES IN MECONOPSIS

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The genus *Meconopsis* (Papaveraceae) consists of 43 species* of which one is confined to western Europe whilst the others are natives of south-central temperate Asia. About twenty species are at present in cultivation, most of these being firmly established, but there are one or two which are difficult to grow and may not persist for long. In addition, a number of hybrids have been described and include some of the most spectacular garden plants belonging to the genus.

Comprehensive taxonomic treatments of *Meconopsis* have been produced this century by Prain (1906), Fedde (1909) and Taylor (1934). Taylor's classification is followed in this account and Table 1 shows it in outline.

The present study was undertaken to determine the chromosome numbers of the species in cultivation at the Royal Botanic Garden, Edinburgh, in the hope that this information would supplement the existing taxonomic knowledge of the genus. Few previous cytological observations have been made; those that exist are summarized in the footnote to Table 2.

MATERIALS AND METHODS

All plants used in this investigation were cultivated in the Royal Botanic Garden, Edinburgh, and voucher specimens have been made for all collections and are lodged in the herbarium there. Many of the collections were originally of known wild origin but in all cases a number of generations have been grown in the garden. It was difficult to demarcate the species boundaries in the *regia–paniculata–napaulensis* group as under garden conditions they seem to be connected by intergrading forms. The plants chosen for cytological investigation are typical of these species according to Taylor's descriptions.

Flower-buds were fixed in 3: 1 alcohol: acetic acid, stored in a 'deep-freeze' at about—17° C and squashed in iron acetocarmine.

The estimates of pollen fertility were made by observing the percentage of grains which appeared well-formed and stained deeply in iron acetocarmine.

RESULTS

The chromosome counts are summarized in Table 2 and illustrated in Plates 10–11 and Figs. 1–8. As shown in the figures the bivalents are generally large, although there is a considerable range in their size. Chiasmata are usually distally localized (see Plate 11A). Meiotic irregularities are rather common in some species. Data for individual species where these are noteworthy are given below. The following abbreviations are used:

P.M.C.	=	Pollen mother cell	M2	=	2nd meiotic metaphase
M1	=	1st meiotic metaphase	A2	=	2nd meiotic anaphase
A1	=	1st meiotic anaphase	T2	=	2nd meiotic telophase
T1	=	1st meiotic telophase			

M. cambrica: The stock studied was growing semi-wild in the 'peat-walls' area of the Royal Botanic Garden, Edinburgh. Thirteen P.M.C. observed at

* 41 species according to Taylor, plus two described since.

diakinesis and M1 showed 14 bivalents (fig. 1). The average chiasma frequency per bivalent at M1 was 1·16. Twelve figures of T1 and M2 had n = 14 at each pole, whilst in a further three figures of M2 there were clearly n = 13 at one pole and n = 15 at the other, indicating that uneven disjunction must have occurred at the previous anaphase.

Table 1

Conspectus of the Species of Meconopsis and their Groups from Taylor 1934. The species in italics have been studied in this investigation.

Subgenus A. MECONOPSIS (=EUMECONOPSIS)

Section I. *CAMBRICAE*

1. *M. cambrica*

Section II. *EUCATHCARTIA*

Series i. Chelidonifoliae
2. *M. chelidonifolia*, 3. M. oliverana

Series ii. Villosae
4. *M. villosa*, 5. M. smithiana

Section III. *POLYCHAETIA*

SUBSECTION A. EUPOLYCHAETIA

Series i. Superbae
6. M. superba, 7. *M. regia*

Series ii. Robustae
8. M. robusta, 9. *M. dhwojii*, 10. *M. gracilipes*, 11. *M. paniculata*, 12. *M. longipetiolata*, 13. M. violacea, *M. napaulensis*

SUBSECTION B. CUMMINSIA

Series i. Simplicifoliae
15. *M. simplicifolia*, 16. *M. quintuplinervia*, 17. M. punicea

Series ii. Grandes
18. *M. integrifolia*, 19. *M. betonicifolia*, 20. *M. grandis*

Series iii. Primulinae
21. M. florindae, 22. M. lyrata, 23. M. primulina

Series iv. Delavayanae
24. M. delavayi

Series v. Aculeatae
25. M. henrici, 26. M. forrestii, 27. M. impedita,
28. M. venusta, 29. M. pseudovenusta, 30. M. georgei,
31. M. lancifolia, 32. *M. horridula*, 33. *M. latifolia*,
34. M. speciosa, 35. *M. aculeata*, 36. M. neglecta,
37. M. sinuata.

Insufficiently known species of Series iii or v—38 M. argemonantha

Series vi. Bellae
39. M. bella

Subgenus B. DISCOGYNE

40. M. discigera, 41. M. torquata

M. villosa: Twenty-six P.M.C. with 16 bivalents were observed at M1 (Plate 10B). The average chiasma frequency per bivalent at M1 was 1·17 .Two P.M.C. at T1 showed n = 16 at the poles.

M. gracilipes: Three specimens were examined. One of these (C 5253) showed regular meiosis but in the other two, (C 5252 and another) meiotic irregularities were common. P.M.C. in the latter plants contained 1–20 unpaired chromosomes at M1 and laggards were present at anaphase. Micronuclei occurred in the latter stages of meiosis and polyspory was observed. Pollen fertility in these plants was reduced to less than 1%.

Table 2

Chromosome Numbers in Meconopsis

	n	Herbarium No.	Pollen fertility
Subgenus EUMECONOPSIS			
Section *CAMBRICAE*			
M. cambrica (L.) Vig.	14, a-c	C 5251	c. 90%
Section *EUCATHCARTIA*			
Series CHELIDONIFOLIAE			
M. chelidonifolia Bur. & Franch.	14	C 1631	8.5%
Series VILLOSAE			
M. villosa (Hook. f.) G. Tayl.	16, d	C 5244	c. 80%
Section *POLYCHAETIA*			
SUBSECTION EUPOLYCHAETIA			
Series SUPERBAE			
M. regia G. Tayl.	28, c	C 1845	—
Series ROBUSTAE			
M. dhwojii G. Tayl.	28	C 5257	90%
M. gracilipes G. Tayl.	28	C 5252	<1%
		(another plant examined, no specimen)	<1%
		C 5253	60%
M. paniculata (D. Don) Prain	28, f	C 5260	—
M. longipetiolata G. Tayl.	28	C 5258	Only 9 apparently viable pollen grains in 10 anthers examined.
M. napaulensis DC	28, g	C 5248	90%
		C 5249	85%
		C 5261	80%
		very variable population, no specimen.	7%
SUBSECTION CUMMINSIA			
Series SIMPLICIFOLIAE			
M. simplicifolia (D. Don) Walp.	41 or 42	C 5375	—
M. quintuplinervia Regel	42	C 5256	80%
M. x cookei (*quintuplinervia x punicea*)	42	C 5250	3-4%
Series GRANDES			
M. integrifolia (Maxim.) Franch.	37	C 5243	—
M. betonicifolia Franch.	41, h	C 5246	17%
		C 5247	98%
		C 5259	95%
		From Rhododendron Walk no specimen	—
	c. 60	C 5254	97%
M. grandis Prain	c. 59	C 1834	8%
Series ACULEATAE			
M. horridula Hook f. & Thoms.	28	C 5242	50%
M. latifolia (Prain) Prain	28	C 5254	80% (in another plant of same population 90%)
M. aculeata Royle	28	C 5255	85%

a n = 14, Sugiura, 1937 and 1940.
b n = 11, Maude, 1940.
c n = 28, Ernst, 1962 and 1965.
d n = 16, Ernst, 1962 and 1965.
e n = 28, Ernst, 1959.
f n = 28, Ernst, 1965 (paniculata ?).
g n = 14, Sugiura, 1940.
h n = 40, Ernst, 1965.

M. longipetiolata: Meiosis was extremely irregular in the stock of this species, consisting of three plants, in cultivation at the Royal Botanic Garden, Edinburgh in 1965. At MI all P.M.C. had many univalents (e.g. configurations of 16_{11} 24_1 and 21_{11} 14_1, Fig. 3). At AI and A2 all P.M.C. exhibited lagging of chromosomes, and following this, supernumerary micronuclei occurred with resultant polyspory. A random sample transect of a preparation at an early stage in pollen grain formation contained the following groupings each derived from one P.M.C.

1) 4 large spores (one with two extra micronuclei) + 2 supernumerary small spores (one binucleate).—2 groupings.
2) 4 large spores + 1 small binucleate supernumerary spore.—2 groupings.
3) Tetrad nuclei + 13 micronuclei, the division into spores seemed to have been disturbed so that only the diad wall had been completed, but an incomplete wall was also visible in one of the diads.
4) 4 large spores, one containing an extra micronucleus.
5) 4 large spores, each with an extra micronucleus.
6) 4 large spores, one containing an extra micronucleus + one small supernumerary spore.
8) 4 large spores, apparently normal.
9) 4 large spores, one containing an extra micronucleus + two small supernumerary spores, one of which was binucleate.

As would be expected, pollen fertility in this stock was negligible, only nine well-formed and deep-staining grains were seen in ten anthers examined. Apparently no seed was set by the three plants in 1965; since the species is monocarpic this resulted in the extinction of the stock.

M. napaulensis: Four stocks of this species were studied. Three showed regular meiosis with 28 bivalents and 80–90% pollen fertility. The fourth stock was notable for its morphological heterogeneity and the single plant examined showed occurrence of univalents at MI, laggards at AI and a pollen fertility of only 7%.

M. simplicifolia: This species has either n = 41 or 42. Although many good figures were examined it was very difficult to be absolutely certain which of these two numbers was correct. At pachytene a few of the P.M.C. showed small heteropycnotic clumps along the chromosome threads but the majority were normal. Quadrivalents, hexavalents and even larger multivalent configurations were common amongst a group of larger chromosomes (Plate IIA-C). The larger associations were very common at diplotene and diakinesis but tended to have dissociated into smaller groupings by MI; Plate IIC, however, shows a P.M.C. with an 8-valent at MI. Anaphase separation and the later stages of meiosis were usually regular but occasional P.M.C. showed lagging of chromosomes and formation of micronuclei.

M. x cookei (*quintuplinervia* × *punicea*): Some P.M.C. in this hybrid exhibited apparently regular chromosome pairing at meiosis but in the majority some univalents were present at MI (fig. 5). The number of these univalents observed

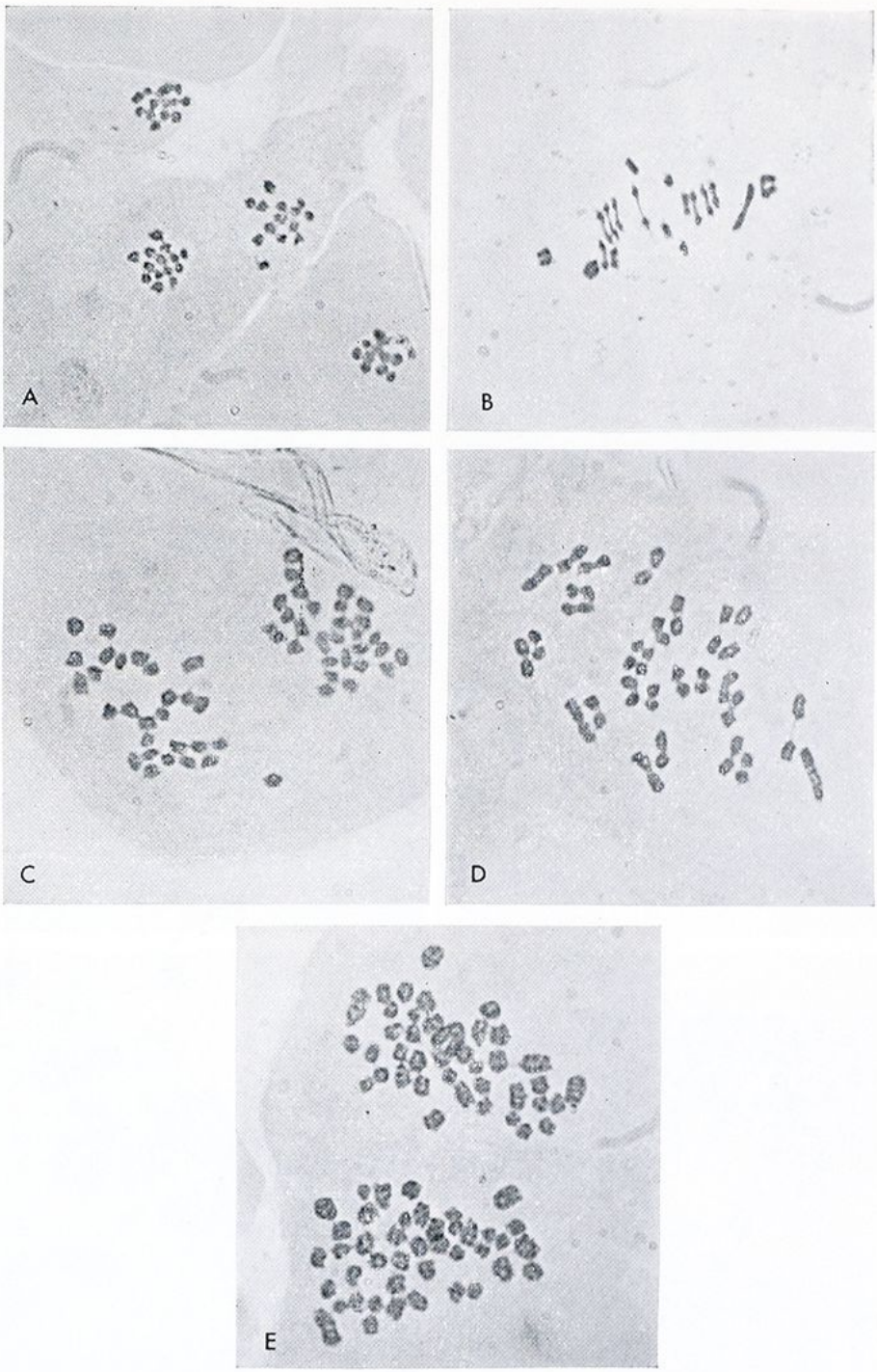


PLATE 10. Meiosis in P.M.C. × 1,000.

A. *Meconopsis chelidonifolia* M2, n = 14; B. *M. villosa* M1, 16 bivalents;
C. *M. dhwojii* T1, n = 28; D. *M. gracilipes* C 5253 M1, 28 bivalents; note secondary associations; E. *M. quintuplinervia* T1, n = 42.

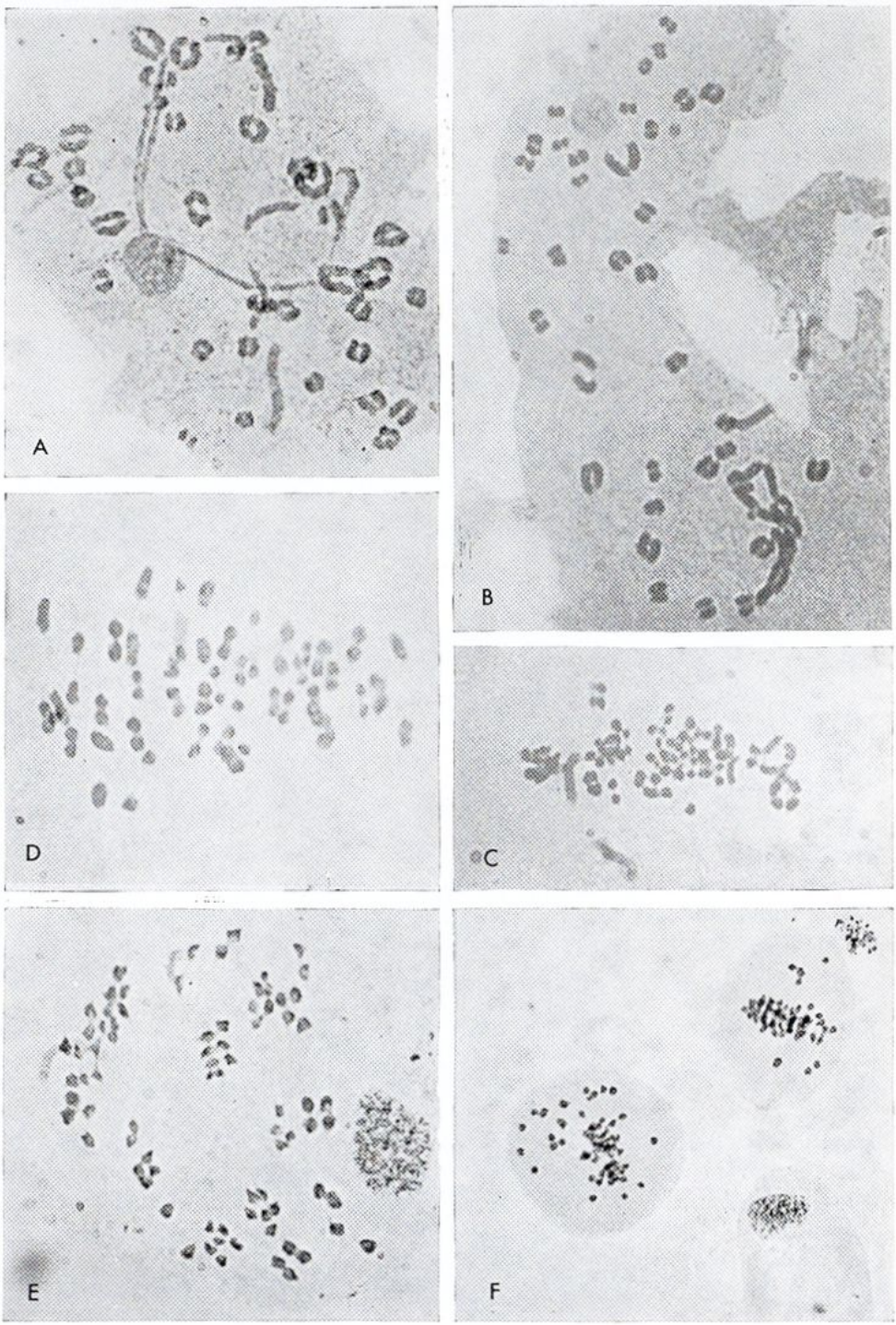
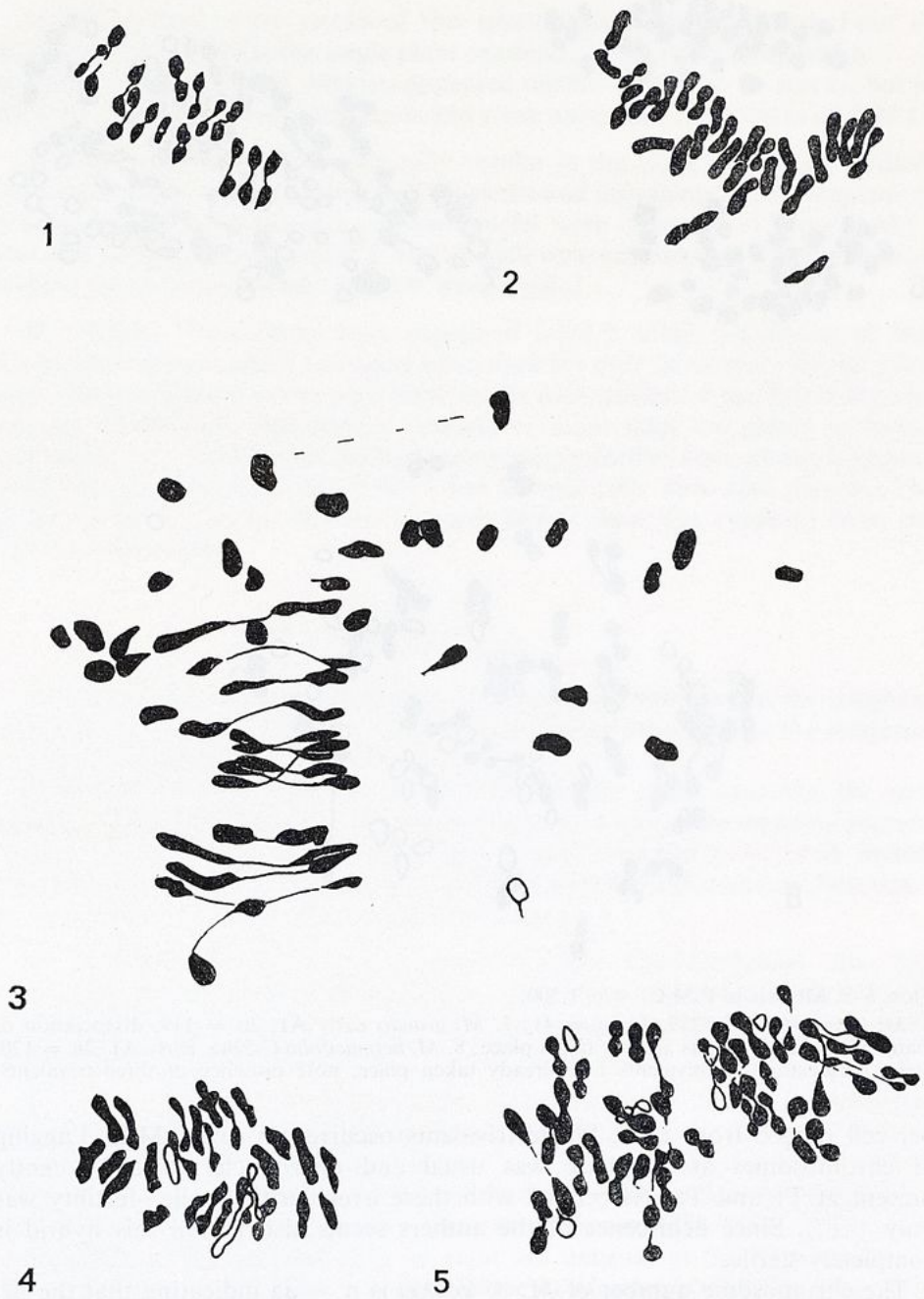


PLATE 11. Meiosis in P.M.C. A–E × 1,000, F × 480.

A. *Meconopsis simplicifolia*, early diakinesis showing very diffuse multivalent;
B. *M. simplicifolia*, later diakinesis showing multivalent at the bottom of the figure;
C. *M. simplicifolia*, M1, 8-valent in figure of 8 configuration at right of figure;
D. *M. integrifolia*, beginning of A1, 37 bivalents some of which have already dissociated;
E. *M. betonicifolia* C 5247, M1, 41 bivalents;
F. *M. latifolia*, M1 showing univalents.



FIGS. 1–5. Meiosis in P.M.C. × c. 1,200.

1. *M. cambrica* M1, 14_{II}; 2 *M. regia* M1, 28_{II}; 3. *M. longipetiolata*, M1 showing bivalents and univalents. Some of the univalents are clearly the result of precocious separation of bivalents; 4. *M. aculeata*, M1, 28_{II}, 3 bivalents have been drawn in outline to increase the clarity of the figure; 5. *M.* × *cookei* beginning of A1 separation. The original configuration was probably $4I_{II}$, 2_I. Some bivalents are drawn in outline as are the 4 unpaired chromosomes (2 of which have separated precociously).

FIGS. 6–8. Meiosis in P.M.C. × c. 1,200.
6. *M. betonicifolia* C 5259, T1, n = 41; 7. *M. grandis* early A1, 2n = 118, dissociation of many of the bivalents has already taken place; 8. *M. betonicifolia* C 5245, early A1, 2n = 120. Some dissociation of bivalents has already taken place, note presence of three trivalents.

per cell ranged from 1–28. Single trivalents occurred in two P.M.C. Lagging of chromosomes at anaphase was usual and micronuclei were frequently present at T1 and T2. Associated with these irregularities pollen-fertility was only 3–4%. Since dehiscence of the anthers seems also to fail this hybrid is completely sterile.

The chromosome number of *M.* × *cookei* is n = 42 indicating that the *M. punicea* parent, which has been lost from cultivation, must have had the same number as *M. quintuplinervia* (i.e. n = 42).

M. integrifolia: Two plants belonging to this species were examined, one in 1964 and the other in 1965. On both occasions many good figures of M1 were seen showing 37 bivalents (Plate 11 d). Some P.M.C. also occurred in which four of the larger chromosomes were associated in a ring quadrivalent.

M. betonicifolia: Five stocks of this species have been examined. Four of these had n = 41 whilst the single plant examined of the other stock had n = ± 60 (plate IIE, figs. 6 & 8). Meiosis appeared regular in all n = 41 stocks, but in the n = ± 60 plant several trivalents and some univalents occurred in all P.M.C.

M. horridula: Meiosis was generally regular in the stock which was studied, but irregularities such as presence of trivalents and univalents at MI, laggards at anaphase and the occurrence of micronuclei were observed in some P.M.C. Out of a sample of twenty-nine P.M.C. which were examined at TI, five showed lagging chromosomes whilst the rest were regular.

M. latifolia: Two plants were examined from a small population of this monocarpic species which has maintained itself for over thirty years by self-sown seed. The population grows on a bank in the rock garden of the Royal Botanic Garden, Edinburgh, and seldom consists of more than ten plants in flower in a single year. Both plants showed meiotic irregularities (univalents, laggards, micronuclei) in many of the P.M.C. but normal cells were also present. The pollen fertility recorded was 80%, much higher than was expected from the meiotic observations.

Discussion

All *Meconopsis* species studied in this investigation belonged to the subgenus *Meconopsis* (= *Eumeconopsis*) since the two species belonging to the subgenus *Discogyne* were unavailable.

In the section *Cambricae* the chromosome number of *M. cambrica*, the only species, was found to be n= 14. This corresponds with counts made by Sugiura (1934, 1940) for this species, but Maude (1940) recorded n = 11 in British material and Ernst (1965) reported n = 28 in material cultivated in America.* n = 14 no doubt represents tetraploidy of a basic x = 7.

M. chelidonifolia (section *Eucathcartia*, series *Chelidonifoliae*) also has n = 14 as in our stock of *M. cambrica*. Henderson (1966) has shown that it also has the same pollen type as *M. cambrica*, so both pollen and cytology indicate affinity between these species.

M. villosa, which Taylor places in the series *Villosae* of section *Eucathcartia*, differs cytologically from all other species investigated. It has n = 16 and therefore seems to be a tetraploid of an x = 8 series, whilst the rest all belong to an x = 7 series or seem to have been derived by descending dysploidy from such a series. As Ernst (1962) points out with reference to *M. villosa*, "Chromosome numbers that are multiples of eight are unusual in the Papaveraceae". The pollen-type of this species is also unique in *Meconopsis* (Henderson, 1966) whilst various other characters are not in accord with the rest of the genus. It is interesting that Hooker originally described the genus *Cathcartia* to accommodate this species and the genus was retained by Prain (1906, 1915) and Fedde (1909). Fedde included other species within the circumscription of *Cathcartia*, as did Prain (1906), but the latter author returned it to its original monotypic state in his revision of 1915. Cytological and palynological evidence support a

* Dr. Ernst has since informed me that this was an error and the report should have read 2n=28.

segregation of *M. villosa* from *Meconopsis* but the recognition of *Cathcartia* as a separate genus is fraught with difficulties as shown by Taylor (1934, p. 5–6).

Five of the seven species belonging to subsection *Eupolychaetia* have been examined and all are characterized by an octoploid number of n = 28. The fact that all these species are octoploid probably indicates that their evolutionary diversification has taken place from an ancestral stock of this high polyploid level.* The three species examined which belong to the subsection *Cumminsia*, series *Aculeatae*, are also octoploids (n = 28). Whether this indicates relationship with the species of the subsection *Eupolychaetia* or merely a parallel occurrence of the octoploid condition is not known. It is, however, interesting to note that the two groups seem to overlap each other in pollen characteristics (Henderson, 1966).

Cytological information is available for all species of subsection *Cumminsia* series *Simplicifoliae* and series *Grandes* and they seem to form a rather natural group. The duodecaploid condition (n = 42) is found in *M. quintuplinervia* and must also characterise *M. punicea* since *M. quintuplinervia* × *punicea* (= *M.* × *cookei*) has n = 42. In *M. simplicifolia* n = either 41 or 42, whilst in four of the stocks of *M. betonicifolia* which were studied n = 41; n = 41 presumably has been derived by dysloidy from an original n = 42 condition. Another stock of *M. betonicifolia* had n = ± 60, whilst the sole stock of *M. grandis* successfully examined had n = ± 59; these numbers are hypo–18–ploids and it is suggested that they may have originally arisen by the union of diploid and haploid germ cells from near duodecaploid parents. *M. integrifolia* with n = 37 stands somewhat apart from the other species but this number may represent further dysploid reduction from a duodecaploid condition. In *M. simplicifolia* multivalent formation is common amongst a group of similar long chromosomes, these are no doubt homoeologous and their association in multivalents indicates that the polyploidy is still relatively 'raw'. Similar groups of long chromosomes have been noted in the other species of this subsection, particularly in *M. integrifolia* where they sometimes form a ring quadrivalent.

These results suggest that in the series *Simplicifoliae* and *Grandes* of subsection *Cumminsia*, speciation has occurred from an ancestral duodecaploid stock. There has been a tendency towards descending dysploidy represented at n = 41 in *M. betonicifolia* and perhaps *M. simplicifolia*, at n = 40 in another stock of *M. betonicifolia* (Ernst, 1965) and at an extreme of n = 37 in *M. integrifolia*. Whether the approximate 18-ploid condition observed in one plant of *M. betonicifolia* and in *M. grandis* is established in nature, or merely represents triploidy (3n = c. 18 x) which has occurred under cultivation is not known.

In a number of the species, irregularities of meiosis occur accompanied by various degrees of loss of fertility. Such irregularities were particularly common in the stocks of *M. longipetiolata*, *M. latifolia* and *M. gracilipes* which were studied. Occurrence of such errors in nature seems unlikely in these monocarpic species and in fact examination showed that the pollen fertility of herbarium specimens of *M. gracilipes* collected in the wild is very high. The irregularities may have arisen under cultivation as a result of excessive inbreeding in small populations; since the morphology of the stocks seems typical for their species, interspecific hybridization is a less likely cause. Very similar meiotic irregularities, attributed to inbreeding, have been described by Shopova (1966) in

* Records of n=14 for *M. napaulensis* and *M. robusta* by Sugiura (1940 and 1944 respectively) may partially vitiate this reasoning.

Capsicum. Rees (1955) has also found characteristic meiotic errors in inbred rye as has Rowlands (1958) in *Vicia faba* exposed to inbreeding.

Pollen fertility of some stocks such as *M. grandis* C. 1834, *M. betonicifolia* C. 5246 and *M. chelidonifolia* C. 1631 are very low although meiotic irregularities have not been observed.

Acknowledgment

I wish to thank Miss H. T. Prentice for her assistance in this research.

Summary

Chromosome numbers have been determined for eighteen species out of a total of forty-three in the genus *Meconopsis*.

Most of the species can be placed in a series based on x = 7, or can more or less readily be derived by dysploid reduction from such a series, but *M. villosa* appears to be based on x = 8 and is therefore discordant.

M. cambrica and *M. chelidonifolia* are tetraploids with n = 14. Five of the seven species belonging to the subsection *Eupolychaetia* have been studied and all are octoploids (n = 28). It is therefore suggested that speciation in this subsection has taken place at the octoploid level. The octoploid condition is also found in the three species of subsection *Cumminsia*, series *Aculeatae*, which were studied. The series *Simplicifoliae* and *Grandes* of subsection *Cumminsia* show either the duodecaploid condition (n = 42) or conditions which can be derived from it by dysploidy or triploidy.

Meiotic irregularities are common in some stocks examined and it is suggested that this is the result of enforced inbreeding under conditions of cultivation.

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